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QUANTITATIVE VASCULAR CASTING OF THE POST-ISCHEMIC HYDRONEPHROTIC KIDNEY

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Abstract

The renal microvasculature (afferent arteriole) and glomeruli were examined and quantitated by two methods in the post-ischemic hydronephrotic (PIH) kidney. The methods used were: 1) an in vivo examination and 2) controlled perfusion-fixation, quantitative vascular casting examined by scanning electron microscopy. The second method was also applied to the vasculature of the contralateral, functional kidney. The goals of the study were to: 1) validate the quantitative vascular casting method by comparing PIH renal microvascular data from the casting method with in vivo values and 2) determine the extent of microvascular dimensional difference of the PIH kidney from its contralateral functional counterpart. It was determined that the casting values were consistent with the data obtained from the in vivo examination of the afferent arteriole and glomeruli. This finding provides further support for the quantitative renal microvascular casting technique. Using that technique it was determined that the dimensions of the microvasculature and glomeruli of the PIH kidney were severely (and significantly, $p<0.05$) reduced compared to its functional mate. Since these PIH vessels show a significant decrement in size, vascular reactivity and functional data based on the PIH vessels should be looked at cautiously. The vasculature and glomeruli of the PIH kidney might not be totally normal, however structurally, the glomeruli do not appear to be dramatically altered.

KEY WORDS: Afferent arteriole, glomerulus, rat, vascular casting, hydronephrosis

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Introduction

The post-ischemic hydronephrotic (PIH) kidney was described by Steinhausen et al. [19] as a way to examine renal microvascular reactivity in vivo. The renal microvasculature plays an important role in the control of renal function. However, studying this vasculature bed has been very difficult. Prior to this PIH method the only way to directly examine renal microvasculature in the animal was with cheek pouch implants of renal tissue [6,15] or using isolated vascular segments which were individually perfused [7-9]. More recently it has also been possible to study the microvasculature of juxtamedullary nephrons in situ of partially dissected isolated perfused kidneys [4-5]. None of these examinations take place on unaltered kidneys, thus their results may not be totally applicable to the microvasculature and glomeruli of the normal (unaltered) kidney. The purpose of the present study is to compare the renal microvasculature in the PIH kidney, which is itself nonfunctional, to the contralateral functional kidney through the use of quantitative vascular casting. This allows a determination of degree of microvascular alteration which has occurred in the PIH kidney compared to its contralateral functional counterpart. The vascular casting values from the PIH kidney are also compared to PIH microvasculature dimensions from in vivo examination. This comparison of in vivo and casting vascular data serve as a way to check the comparability of the quantitative casting method to the real values of the in vivo kidney, even though that kidney might not be totally normal.

Materials and Methods

The post-ischemic hydronephrotic kidney operation was performed on male Wistar rats at approximately seven weeks of age. The operation was performed under sodium pentobarbital (50 mg/kg) anesthesia and consisted of a left subcostal flank incision through which the ureter was completely ligated while the renal artery was temporally ligated (60 minutes) after which the incision was closed in two layers, a) peritoneum and muscle, and b) skin. The animals were allowed to survive for 35-40 days postoperatively. The kidneys of selected animals were perfusion fixed before this time for a light microscopic

study of the intrarenal alterations which were occurring; times included 1 day and 10 days. The animals which were allowed to fully develop the PIH kidney were divided into two groups: a) those whose kidneys were to be perfusion fixed and cast, and b) those whose kidneys were to be examined in vivo. The methods utilized with the first group are described in detail elsewhere [11-13], but briefly consisted of; a) anesthesia (sodium pentobarbital, 50 mg/kg), b) laparotomy (on warmed table) with insertion of abdominal aortic catheter (for blood pressure determination and perfusion fixation and casting), c) ligation of superior mesenteric artery and suprarenal abdominal aorta with incision of inferior vena cava, d) controlled perfusion fixation (brief saline flush followed by 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, 38°C at physiologic pressure, about 120 mmHg in each animal), and e) casting of renal microvasculature with Batson's compound No. 17 (Polysciences Inc., Warrington, PA). The cast kidneys were allowed to sit in saline overnight at room temperature to allow the plastic to completely polymerize and tissue digested with 30% KOH. The casts were washed in several changes of distilled water and allowed to air dry. Segments were mounted on stubs with silver paste, sputter coated with gold and examined and quantitated at 15 kV with an AMR 1200 scanning electron microscope. Precautions were taken (as described elsewhere [11-13]) to insure the calibration of the microscope was correct. Proximal (@ 100 µm from glomerulus) and distal (<20 µm from glomerulus) afferent arteriolar diameters (PAD and DAD respectively) were taken as well as glomerular tuft diameters in two perpendicular directions (equatorial as well as vascular pole to urinary pole) from both the PIH kidney cast as well as from the contralateral kidney. The afferent arteriolar and glomerular dimensions from the contralateral kidney were taken predominantly from outer cortex but did include measurements from all cortical regions since, a) cortical location of origin for PIH values could not be determined, and b) presumably most of the remaining glomeruli in the PIH kidney would have been outer cortical since there is more selective loss of inner cortical glomeruli [17]. The mean values per region (PAD, DAD, glomerular diameter, 20 each) per kidney per animal were compared with their respective contralateral value by paired t-test analysis.

Those kidneys which were to be studied in vivo were studied by a modified Steinhausen [19] method which included: a) anesthesia (sodium pentobarbital, 50 mg/kg), b) laparotomy on warmed table with gentle isolation of the PIH kidney, c) splitting the kidney along the greatest (lateral) margin with an electrocautery knife, d) gently spreading the thinned renal wall with forceps, and e) examination with Wild 420 photomacroscope (32X zoom magnification with 2X extender and 20X photo eyepiece) with photography onto Kodak VR1000 color film. Photographs were also taken of a stage micrometer for calibration of final print magnification. Afferent (30-80 µm from glomerulus) arteriolar and glomerular diameters were measured from the prints and compared to the casting values by unpaired t-test. After the photomacrography, the animals' kidneys were perfusion fixed with 2.5% buffered glutaraldehyde for light and scanning electron microscopic examinations.

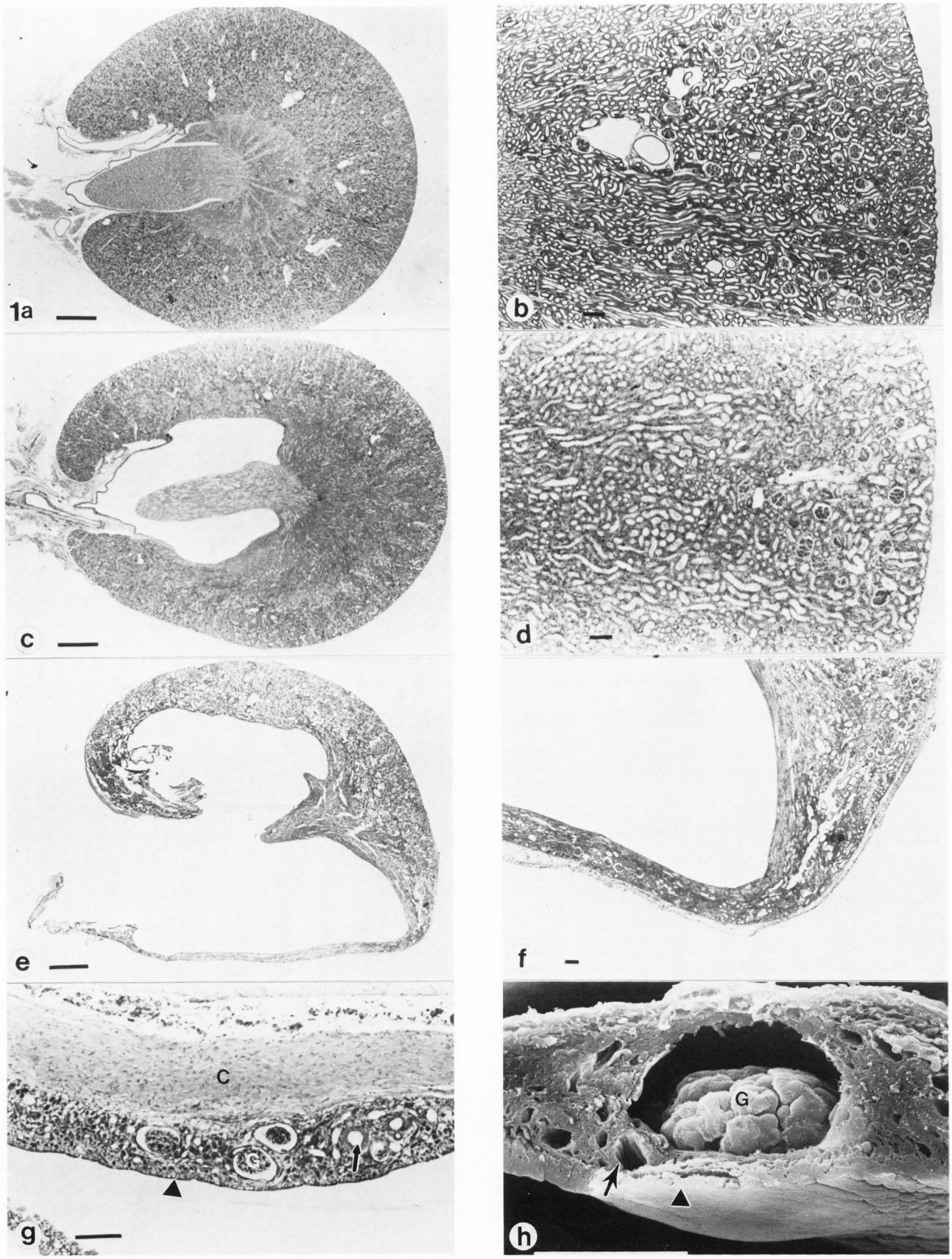
Light microscopic preparation of fixed tissue included standard paraffin processing and sectioning. Sections were stained by the periodic acid Schiff reagent (PAS) method and photographed with the Wild 420 photomacroscope. For SEM examination of glomeruli, segments of renal wall of PIH kidney and cortex of the contralateral kidney were rinsed, postfixed in 1% OsO_4, rinsed and dehydrated through a graded series in ethanol. In absolute ethanol, segments of tissue were frozen in liquid nitrogen and cryofractured prior to critical point drying. Dried tissue was mounted on double stick tape and silver paint used to insure tissue conductance prior to sputter coating with gold. The tissue was examined at 30 kV with an AMR 1200 SEM; photographs were taken with Polaroid type 55 P/N film.

Results

Development of the PIH kidney

In the PIH kidney, one day after the renal ischemia was induced, the renal parenchyma shows alterations (Figs. 1c and d) compared to non-ischemic controls (Figs. 1a and b). Some tubules are collapsed and exhibit some cell swelling. At this time, the renal pelvis is already dilating from the ureter ligation (Fig. 1c). By 10 days, the most pronounced changes are the dilated renal pelvis and thinned anterior and posterior renal

Figure 1. Development of the PIH kidney. a,b. Light micrographs of contralateral control kidney of operated rat with normal cortex, outer and inner medullae. Bar = a. 1 mm; b. 500 µm. c,d. Light micrographs of a kidney 1 day after PIH operation. Dilation of the renal pelvis from the ureter ligation is also evident. There is some tubular change noted with apical blebbing and some sloughing of the epithelia. There is, as yet, no real thinning of the renal cortices. Bar = c. 1 mm, d. 500 µm.
e,f. Light micrographs of a kidney 10 days after PIH operation. There is marked dilation of the renal pelvis with prominent atrophy of the cortex and medulla, notably of the anterior-posterior wall. Evidence of loss of tubules is noted. The thinned regions of renal wall are comprised of blood vessels glomeruli and some tubular components. Bar = e. 1 mm; f. 500 µm.
g. Light micrograph of renal wall from fully developed PIH kidney. The renal wall possesses a thickened capsule (C), urinary epithelium along the pelvic aspect (arrowhead), glomeruli (G), and blood vessels (arrow). Tubular components are not seen. Bar = 100 µm.
h. Scanning electron micrograph of the renal wall from a fully developed PIH kidney showing its components. Bar = 100 µm; urinary epithelium (arrowhead), glomerulus (G) and blood vessels (arrow).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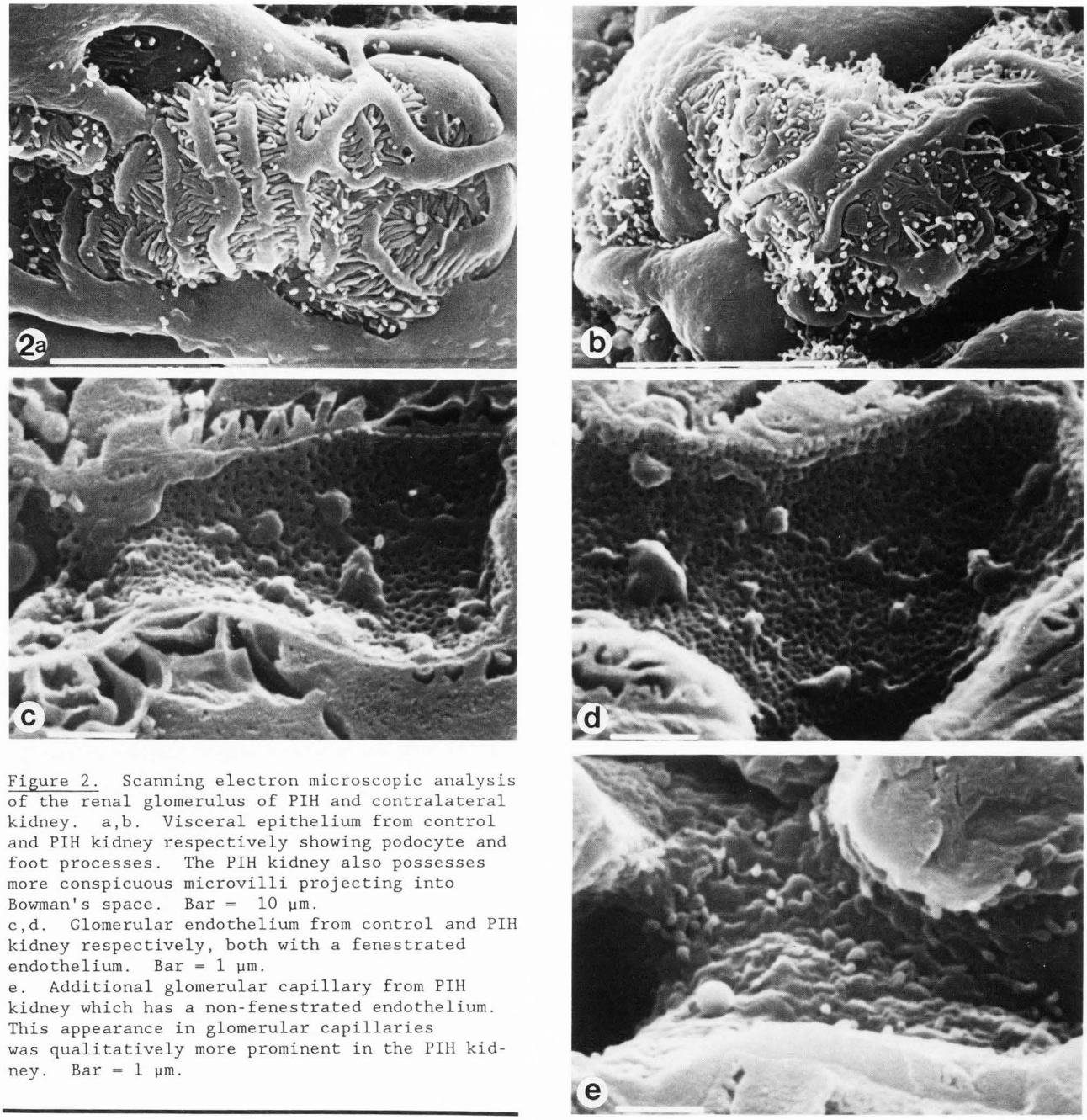


Figure 2. Scanning electron microscopic analysis of the renal glomerulus of PIH and contralateral kidney. a,b. Visceral epithelium from control and PIH kidney respectively showing podocyte and foot processes. The PIH kidney also possesses more conspicuous microvilli projecting into Bowman's space. Bar = 10 µm.
c,d. Glomerular endothelium from control and PIH kidney respectively, both with a fenestrated endothelium. Bar = 1 µm.
e. Additional glomerular capillary from PIH kidney which has a non-fenestrated endothelium. This appearance in glomerular capillaries was qualitatively more prominent in the PIH kidney. Bar = 1 µm.

cortical walls (Fig. 1e). At this time, tubular atrophy and/or loss are present throughout the thinned walls (Fig. 1f). In the mature PIH kidney, these thinned renal walls contain mainly glomeruli and vascular elements (Figs. 1g and h). The glomeruli appear smaller in the mature PIH kidney compared to its functional contralateral kidney.

Morphology of the glomerulus in PIH kidney

By scanning electron microscopy the glomerular visceral epithelium and endothelium of the PIH kidney (Fig. 2b and d) are not drastically different from that of the contralateral functional counterpart (Figs. 2a and c). The visceral epithelial cell possess foot processes from both kidneys (Figs. 2a and b), however in the PIH kidney these cells also possess scattered microvilli. The glomerular endothelium possesses numerous fenestrae in both kidneys (Figs. 2c and d), however in the PIH kidney there are also many capillary segments which appear to be non-fenestrated (Fig. 2e).

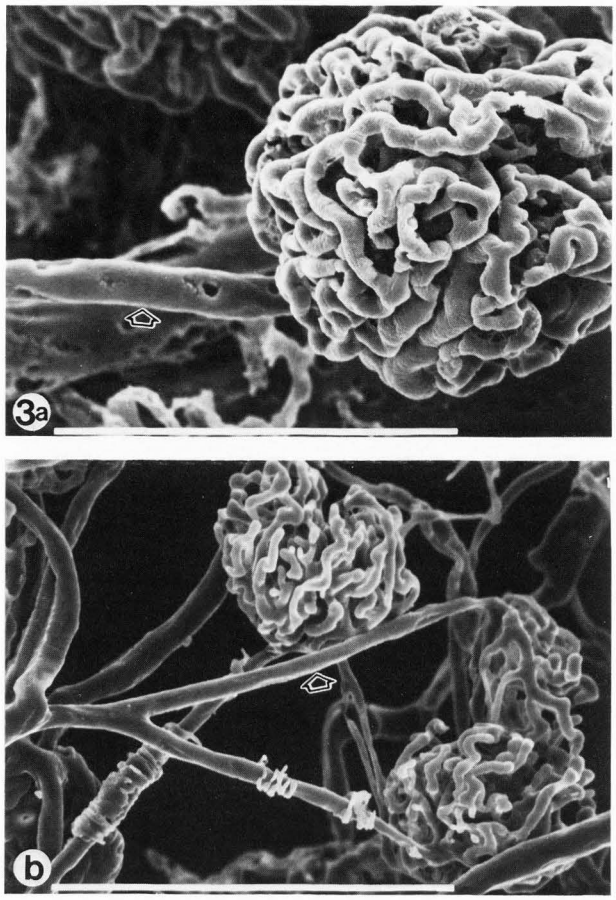


Figure 3. Scanning electron micrograph of vascular casts with afferent arteriole (arrow) and glomerulus from control (a) and PIH (b) kidneys. Note the smaller diameter of the afferent arteriole and glomerulus from the PIH cast. The glomerular capillary loops appear to be thinner in the PIH kidney as well. Bar = 100 µm.

Table 1

Quantitative Data on Renal Microvasculature

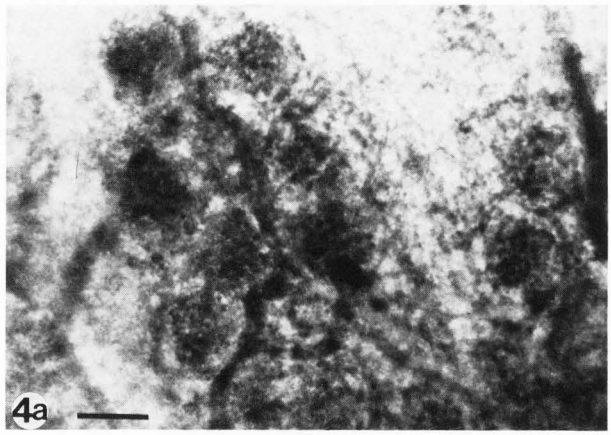
(mean, µm ± sem)

	Casting		In Vivo
	PAD	DAD	
Afferent Diameter			
PIH Kidney	8.4 ± 0.3	5.6 ± 0.2	6.7 ± 0.2
Contralateral Kidney*	17.9 ± 2.7	14.3 ± 1.8	
Glomerular Diameter			
PIH Kidney+		84.1 ± 3.9	95.6 ± 2.5
Contralateral Kidney*		140.5 ± 26.8	

*Difference ($p<0.05$) for casting values between PIH and contralateral kidneys by paired T test.
+No difference ($p>0.05$) between casting and In Vivo analysis data.
N=3 per group

Quantitative vascular casting and in vivo study of PIH kidney

The afferent arteriolar and glomerular diameters appear smaller from the casts of the PIH (Fig. 3a) compared to the functioning contralateral kidney (Fig. 3b). Quantitative analysis of the proximal (PAD) and distal (DAD) afferent arteriolar diameter as well as glomerular diameters are smaller ($p<0.05$ by paired t test) in the PIH than its contralateral counterpart (Table 1). It also appears that the glomerular capillary loops themselves have a smaller diameter in the PIH kidney. The glomerular appearance by casting is strikingly similar to that seen in the in vivo kidneys (Figs. 4a and b) and their size was not different by the two methods. The vessels and glomeruli are more clearly seen on an undigested segment of cast PIH kidney (Figs. 5a and b). The microvascular values for the PIH afferent arterioles from in vivo analysis is intermediate between the PAD and DAD from the casting study which is appropriate for where the values were obtained.



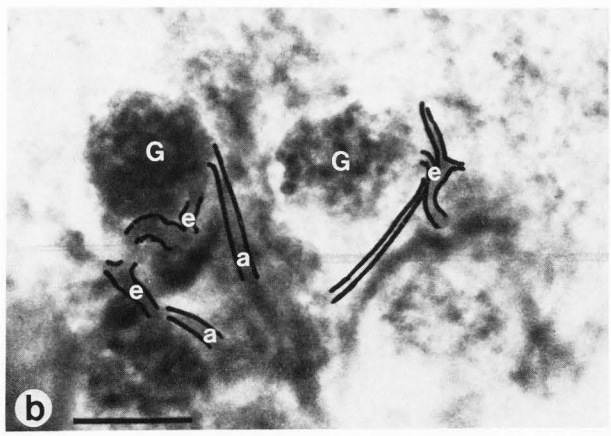


Figure 4. Photomicrography of the PIH kidney from the in vivo preparations. The use of color film helps accentuate the vessels and glomeruli by visualization of the red blood cells, therefore in the black and white copy (a and b), the afferent (a), and efferent (e) arterioles are outlined as they enter and exit from their associated glomerulus (G). Bar = 100 µm.

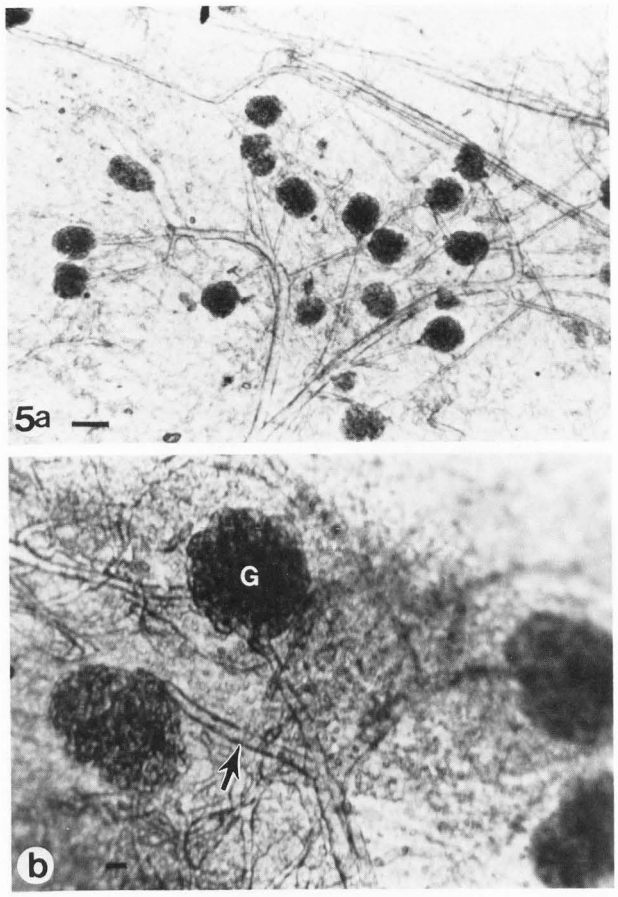


Figure 5. Photomicrography of renal wall of a PIH kidney after perfusion fixation and casting. The vascular arrangement is more clearly seen with the afferent arterioles (arrow) and glomeruli (G). Bar = a. 100 μm; b. 10 μm.

Discussion

The present study has demonstrated and quantitated with a vascular casting technique a renal vascular and glomerular dimensional difference in the post-ischemic hydronephrotic (PIH) kidney compared to its functional contralateral counterpart. The casting values for the afferent arteriole and glomerulus of the PIH kidney are in close agreement with values obtained by in vivo examination of these structures. This finding indicates that the quantitative casting method provides renal vascular dimensional values which accurately represent their "real" values.

Other methods used to examine and quantitate renal vascular dimensions usually need to alter or disrupt aspects of the renal architecture. The transplantation of developmental renal tissue into hamster cheek pouch [6,15] places kidney tissue into a foreign location so that the vascular supply and drainage, and probably innervation are not normal. The post-ischemic hydronephrotic kidney preparation has, as this study has demonstrated, abnormal vascular dimensions. This point is conceded by Steinhausen et al. [19] in the initial description of the PIH kidney by acknowledging lower renal blood flow, however the degree of vascular change was not known. Since these vessels and glomeruli are already significantly reduced in size, it might be difficult to clearly define differences in renal vascular reactivity for normal renal vasculature based on these abnormal vessels. However, it is known that the PIH kidney vessels are innervated [3]. Another method to examine renal microvasculature is to remove and partially dissect the kidney, and then perfuse it in vitro while examining the vasculature of the juxtamedullary nephrons [3,4]. These are normal renal vessels, at this point denervated, which gives specific insight into the vessels of juxtamedullary nephrons. Isolated vessels (afferent and efferent arterioles) from outer cortical nephrons can be individually perfused and viewed although the method is technically difficult [7-9]. All of these methods have their individual advantages, however, they all have drawbacks if you are attempting to understand renal vasculature in the unaltered kidney in situ. The quantitative renal vascular casting method as used herein does not allow the examination of the dynamics of renal vascular reactivity as is possible in these other preparations. However, the method does allow the examination and quantitation of the renal vasculature from the intact kidney from which juxtamedullary and regional cortical vascular dimensions can be individually obtained. Since the kidneys were fixed and cast in situ, they would have had their normal innervation which could be important in the discrimination of neurally mediated or modulated vascular changes from humoral or structural changes. The comparison of the casting and in vivo vascular dimension data from the study herein supports the validity of the casting method to accurately represent the functional state of the renal vasculature at the time of fixation and casting.

The intrarenal vascular changes seen in the PIH kidney are themselves very interesting. The glomeruli appear to diminish in diameter, at least compared to their contralateral counterparts. It is unknown if the afferent arteriolar changes occur at the same time as the glomerular changes. However, it should be noted that a decreased glomerular size has also been described in glycerol-induced acute renal failure [10] and the 5/6 nephrectomy model of chronic renal failure [18]. Some of these renal and glomerular changes may be important in the decrement of renal function. Recent work [1,16] has shown that angiotensin converting enzyme inhibitors allow the glomerulus to perfuse and function fairly normally in the chronic renal failure models. Since the glomerulus is the major renal site of angiotensin II receptors [14] which can cause glomerular mesangial contraction [2], AII might be mediating this decrease in glomerular size [20]. However, very recent work with the PIH kidney would seem to contradict this hypothesis [21].

The vasculature and glomeruli of the contralateral kidney of the PIH animal is functional and has undergone compensatory hypertrophy. The vascular and glomerular dimensions for this kidney may not be totally normal as well, however, their sizes appear to be more in line with the expected values from prior casting studies with Wistar (Kyoto) rats [11] and Sprague-Dawley rats [12-13].

In conclusion, the present study indicates that, 1) the renal microvasculature and glomerulus of the PIH kidney are smaller than one would normally expect. This vascular change (of unknown etiology) might indicate the use of some caution when interpreting vascular reactivity data from the PIH model. 2) The comparison of casting vascular values with the in vivo values for the PIH kidney provides support for the quantitative renal vascular casting methodology.

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References

1. Anderson S, Rennke HG, Brenner BM. (1986) In arresting progressive renal disease, all anti-hypertensive drugs are not created equal. Kidney Int. 29; 314.
2. Ausiello DA, Kreisberg JI, Roy C, Karnovsky MJ. (1980) Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. J. Clin. Invest. 65; 754-760.
3. Buhrle CP, Nobling R, Taugner R. (1985) Intracellular recordings from renin-positive cells of the afferent arteriole. Am. J. Physiol. 249; F272-F281.
4. Casellas D, Navar LG. (1984) In vitro perfusion of juxtamedullary nephrons in rats. Am. J. Physiol. 246; F349-F358.
5. Casellas D, Carmines PK, Navar LG. (1985) Microvascular reactivity of in vitro blood perfused juxtamedullary nephrons from rats. Kidney Int. 28; 752-759.
6. Click RL, Joyner WL, Gilmore JP. (1979) Reactivity of glomerular afferent and efferent arterioles in renal hypertension. Kidney Int. 15; 109-115.
7. Edwards RM. (1983) Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. Am. J. Physiol. 244; F526-F534.
8. Edwards RM. (1985) Effects of prostaglandins on vasoconstrictor action in isolated renal arterioles. Am. J. Physiol. 248; F779-F784.
9. Edwards RM. (1985) Response of isolated renal arterioles to acetylcholine, dopamine and bradykinin. Am. J. Physiol. 248; F183-F189.
10. Gattone VH, Evan AP, Luft FC. (1982) The afferent arteriole in acute renal failure. Clin. Res. 30; 448A.
11. Gattone VH, Evan AP, Willis LR, Luft FC. (1983) Renal afferent arteriole in the spontaneously hypertensive rat. Hypertension 5; 8-16.
12. Gattone VH, Evan AP, Mong SA, Connors RA, Aronoff GR, Luft FC. (1983) The morphology of the renal microvasculature in glycerol- and gentamicin-induced acute renal failure. J. Lab. Clin. Med. 101; 183-195.
13. Gattone VH, Evan AP. (1986) Quantitative renal vascular casting in nephrology research. Scanning Electron Microsc. 1986;I:253-262.
14. Gelhart DR, Speth RC and Wamsley JK. (1984) Autoradiographic localization of angiotensin II receptors in the rat brain and kidney. Europ. J. Pharmacol. 98; 145-148.
15. Gilmore JP, Cornish KR, Rogers SD, Joyner WL. (1980) Direct evidence for myogenic autoregulation of the renal microcirculation in the hamster. Circ. Res. 47; 226-230.
16. Jackson B, Dekrevi L, Cubela R, Whitty M, Johnson CI. (1986) Renal function preservation by blood pressure reduction in the rat remnant kidney model of chronic renal failure. Kidney Int. 29; 320.
17. Kallskog O, Hellstrom I, Rissler K, Wolgast M. (1985) Long-term recovery of superficial and deep glomeruli after acute renal failure evoked by warm ischemia. Renal Physiol. 8; 328-337.
18. Kenner CH, Evan AP, Blomgren P, Aranoff GR, Luft FC. (1985) Effect of protein intake on renal function and structure in partially nephrectomized rats. Kidney Int. 27; 739-750.
19. Steinhausen M, Snoei H, Parekh N, Baker R, Johnson PC. (1983) Hydronephrosis: A new method to visualize vas afferens, efferens and glomerular network. Kidney Int. 23; 794-806.
20. Weinberger HD, Martin BA, Gattone VH, Evan AP, Connors BA, Luft FC, Willis LR. (1986) The effect of angiotensin II on glomerular function and morphology in the rat. J. Submicr. Cytol. 18; 29-34.
21. Wiegman DL, Weis S, Dussel R, Steinhausen M. (1985) Angiotensin blockade at eight vascular levels in the rat microcirculation. The Physiologist 28; 268.

Discussion with Reviewers

D.B. Jones: Have you compared the glomeruli and artery size with control kidneys prepared in the same manner? The reduction in functioning renal mass from the loss of function of the PIH kidney might result in hyperperfusion of the contralateral kidney, thus resulting in a supranormal glomerular size.

R.M. Edwards: Do the authors have any vascular and glomerular dimensions from normal rats in order to compare with the PIH and contralateral kidneys (which has hypertrophied), i.e., how different are the dimensions of the PIH kidney compared to normal kidneys?

Authors: In this study we have not compared glomerular or arteriolar size with sham operated controls. While the size of glomerular and arterioles in the contralateral functional kidney are consistent with values for Sprague-Dawley [10] and Wistar Kyoto rats [11], we cannot exclude the possibility of a vascular or glomerular change with its compensatory hypertrophy.

R.M. Edwards: What are the potential reasons and functional consequences of the smaller glomerular and arteriolar dimensions in the PIH kidney?
Authors: The smaller vessels and glomeruli could be due to either; a) contraction (vasoconstriction or mesangial contraction initiated possibly under extrinsic influence, i.e., AII) or b) structural reorganization (an intrinsic vascular or glomerular alteration). The real explanation is, as yet, unknown. The smaller vascular dimension would raise vascular resistance, otherwise I could not speculate on any functional consequence of the changes since the PIH kidney is non-functional anyway.

R.M. Edwards: If vascular reactivity refers to responsiveness to vasoactive agents, why would changes in vascular dimensions alter responsiveness? I would think that changes in receptor density/distribution and/or post-receptor events would be more important.
Authors: If there are alterations of the vascular smooth muscle either structurally or in its normal tone (i.e., from chronic vasoconstriction), it would not seem implausible that changes might have also occurred in receptor (density/distribution) or excitation - contraction coupling. While responsiveness of these vessels to exogenous agents may be totally normal, I believe that corroborative evidence from preparations like yours [7-9] would be necessary to make an assumption that these vessels are normal.

T. Murakami: The authors described that the dimensions of the glomeruli of the PIH kidney were severely reduced. Did such kidney glomeruli with reduced dimensions contain reduced or small numbers of the glomerular capillaries?
Authors: While we did not quantitate either the number or diameters of the glomerular capillaries, it was our impression that virtually all their diameters and in some glomeruli, their numbers were reduced.

D. Casellas: Unlike the glomerulus depicted in Figure 3a the PIH glomeruli of Figure 3b are partially filled (i.e., numerous blind-ended glomerular capillary casts). Does this reflect a "nonfunctional" state of PIH glomeruli or simply a viscosity-related artefact?
T. Murakami: Many interruptions or discontinuities of the cast glomerular capillaries are observed (Fig. 3a, b). No efferent arteriole of the glomerulus was cast (Fig. 3a, b). Please explain the reasons why they are.
Authors: These were difficult to cast simultaneously since there was an apparently greater vascular resistance in the PIH compared to its contralateral kidney. This difference in vasculature was accentuated by the infusion of the viscous plastic. Therefore, there was a real tendency to overcast (filling too much of the peritubular capillary venous system) the contralateral while undercasting (not filling all the glomeruli and arterial system) the PIH kidney. Virtually all glomeruli of the contralateral kidney had cast efferents and peritubular capillaries, sometimes requiring some dissection to better visualize the arterial tree. Most of the glomeruli of the PIH kidney had cast efferent arterioles, however for some glomeruli a few of the capillaries and the efferent arteriole would not be cast. This was probably related to the viscosity of the Batson's compound.

D. Casellas: Figure 1e shows a dramatic reduction in cortical thickness with PIH. Yet Figure 5a shows interlobular arteries endowed with numerous glomeruli and juxtamedullary ones are visible on the upper part of that figure. Have the authors any idea about the number of glomeruli in PIH kidneys? And were there any arterio-venous shunts or aglomerular arterioles that might have reflected glomerular degeneration in PIH?
Authors: We don't have any data on the number of glomeruli, but would guess that there is a reduction. Because of the radically altered architecture of these kidneys, it was not possible to define AV shunts or aglomerular arterioles.

D. Casellas: Were there any vascular (diameter) changes noted at the level of arcuate, interlobular and more importantly efferent arterioles in PIH kidneys?
Authors: It is very difficult to quantitate arcuate or interlobular diameters in normal kidneys due to lengths and lack of a reference point for uniform data collection. Since the architecture was so distorted in these PIH kidneys, collection of that data was not even attempted. It should be possible to quantitate efferent arterioles however this too, is difficult (see brief discussion of this in [13]) and was not attempted here.